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TECHNICAL MANUSCRIPT 578

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William Johnson

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 578

STUDIES OF RIBOSOMAL ANTIGENS FROM PASTEURELLA PESTIS

William Johnson

Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 18662706A072

January 1970

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Several laboratories have shown that ribosomal fractions isolated from four virulent species of bacteria protect animals against challenge with the homologous live virulent organism. To examine the efficacy of using these procedures to prepare protective antigens against Pasteurella pe tis, ribosomes were isolated from mechanically disrupted virulent P. pestis strain Kim 10. RNA was isolated by both ethanol precipitation and phenol extraction of ribosomes. Ribosomal protein was extracted with acidified 2-chloroethanol. Both the RNA and protein preparations were free of W and Fraction I antigens. Ribosomal antigens were injected into animals in doses ranging from 10 to 500 μ g with Freund's complete adjuvant. In general, none of the ribosomal antigens protected satisfactorily against challenge doses of 300 LD₅₀ of P. pestis. Less than 10% of the animals survived challenge. Sera from animals that survived challenge after immunization with ribosomal proteins contained antibodies to both the ribosomal protein and Fraction I. Animals surviving challenge after immunization with ethanol-precipitated RNA produced antibodies to both the RNA preparation and Fraction I. However, the antibodies against the RNA preparation were directed against the protein moieties and not RNA. Phenol-extracted RNA failed to elicit any antibody response but animals surviving challenge after immunization with this RNA preparation did have antibodies to Fraction I.

STUDIES OF RIBOSOMAL ANTIGENS FROM PASTEURELLA PESTIS*

Recent reports have indicated that ribosomal antigens from some species of Staphylococcus,¹ Salmonella,^{2,3} Pseudomonas,³ and Mycobacterium⁴ are effective in immunizing animals against challenge with the homologous live virulent organism. In our laboratory, we have isolated ribosomal RNA and protein from fully virulent cultures of Pasteurella pestis and have examined these macromolecules as potential protective antigens against infection with P. pestis.

Cultures of P. pestis were grown at 26°C in a medium containing 3% N-Z-Amine, 2.5×10^{-3} molar sodium thiosulfate, and 1% xylose. Cells were harvested by centrifugation, suspended in tris buffer containing 10^{-2} molar magnesium acetate, 0.5% Brij 58, and 2 µg of DNase per ml, and were broken in either a French press or an ultrasonicator. Ribosomes were isolated by ammonium sulfate precipitation and washed 10 times in tris buffer containing 10^{-2} molar magnesium acetate.⁵

Ribosomal proteins were extracted with cold acidified 2-chloroethanol.⁶ The procedure for washing the ribosomes was found to be critical for the preparation of pure ribosomal fractions. Proteins extracted from ribosomes washed less than seven times had pesticin activity and were lethal for mice in quantities of more than 50 µg. The lethality of these preparations was due to the presence of small amounts of murine toxin.

Two preparations of ribosomal RNA were isolated. One was obtained by the direct precipitation of ribosomes with ethanol and contained 10 to 15% protein. The other RNA preparation was obtained by phenol extraction of ribosomes and contained no detectable protein. Each antigen was mixed with an equal volume of Freund's complete adjuvant and injected subcutaneously in 0.2-cc amounts.

The ribosomal protein preparation was free from Fraction I and VW antigens, as shown in Figure 1. The center well contained the ribosomal protein. The first well contained a commercial preparation of anti-plague serum, the second well contained adsorbed anti-VW serum, the third well contained anti-Fraction I and the fourth well contained antiserum prepared in mice against ribosomal proteins. There were no reactions between the ribosomal protein and anti-Fraction I, anti-VW, or commercial antiserum. Precipitin bands were observed only between the ribosomal protein and anti-ribosomal protein.

Figure 2 shows the results of acrylamide gel electrophoresis of the ribosomal proteins.⁷ At least 18 bands have been observed. Acrylamide gel immunoelectrophoresis also failed to detect either VW or Fraction I antigens in the ribosomal protein preparation.

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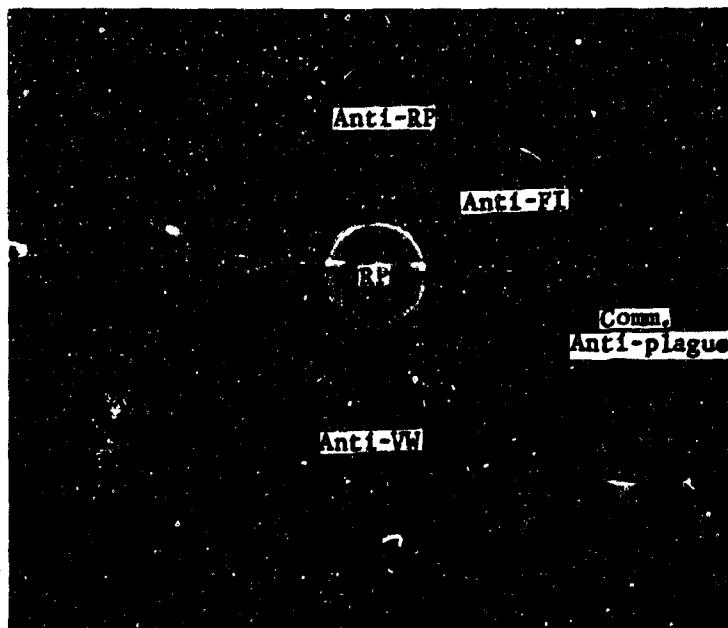


FIGURE 1. Ouchterlony Gel Diffusion of Purified Ribosomal Protein.

Acrylamide Gel
Electrophoretic
Pattern of
Ribosomal Proteins

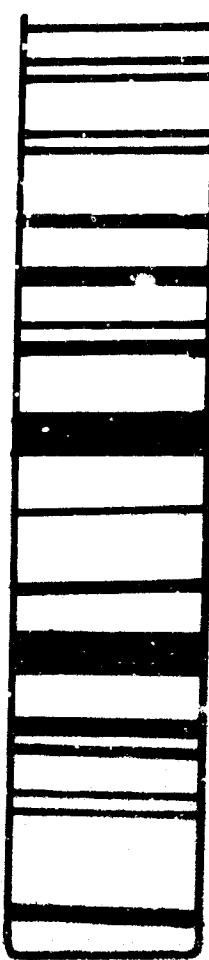


FIGURE 2. Acrylamide Gel Electrophoretic
Pattern of Ribosomal Proteins.

Table 1 is a compilation of four experiments on animals injected with varying amounts of ribosomal protein. Each group was challenged with 300 LD₅₀ of *P. pestis*. The few animals surviving the challenge dose produced antibodies not only to the ribosomal protein, but also to Fraction I and live Kim 10. One of the survivors also had antibodies to acetone-dried *P. pestis* strain Alexander.

TABLE 1. RESPONSE OF MICE TO IMMUNIZATION WITH RIBOSOMAL PROTEIN

Amount Injected, μg	Dead/ Injected	Ribosomal Protein	Antibodies to		
			Fraction I	Kim 10	Alex.
200	39/40	+	+	+	-
100	40/40	0	0	0	0
50	38/40	+/+	+/+	+/+	+-
25	40/40	0	0	0	0
0	40/40	0	0	0	0

Figure 3 shows a typical Ouchterlony gel diffusion analysis of the serum from one of the mice surviving after immunization with purified ribosomal protein. (Two peripheral wells were not used.) Antibodies were produced to Fraction I, ribosomal protein, and Kim 10. No antibodies were detected against acetone-dried strain Alexander.

Table 2 shows the results of immunization attempts with ethanol-precipitated ribosomes and is a compilation of results. In each group, 10 animals were injected with 200, 100, 50, or 25 μg of protein of the ribosome preparation. The first group of animals received one immunizing dose. With the second group, we attempted to determine whether or not the ribosome preparation would produce a rapid, nonspecific resistance to infection as has been described for other RNA. In this group, the animals were challenged 24 hours after administration of the antigen. The third group received two injections 7 days apart. The surviving animals produced antibodies to the ribosome preparation and Fraction I.

Figure 4 shows the gel diffusion pattern of serum from the six animals that survived challenge after immunization with ethanol-precipitated ribosomes. All sera showed lines of precipitation against the ribosomal antigen. Figure 5 shows that the sera from mouse 2 had no detectable antibodies to Kim 10 or Alexander. Mice 3 and 6 had antibodies only to Kim 10. The rest of the mice had antibodies to both Kim 10 and Alexander.

TABLE 2. RESPONSE OF MICE TO IMMUNIZATION WITH AN ETHANOL-PRECIPITATED RIBOSOMAL VACCINE

Injection Schedule	Dead/ Injected	Antibodies to	
		E-P R ^{a/}	Fraction I
One Injection (challenged on day 14)	39/40	+	+
One Injection (challenged on day 2)	38/40	+/-	+/-
Two Injections, 7 days apart (challenged 14 days after second injection)	37/40	+/-/+	+/-/+
Control	39/40	-	+

a. Ethanol-precipitated ribosomes.

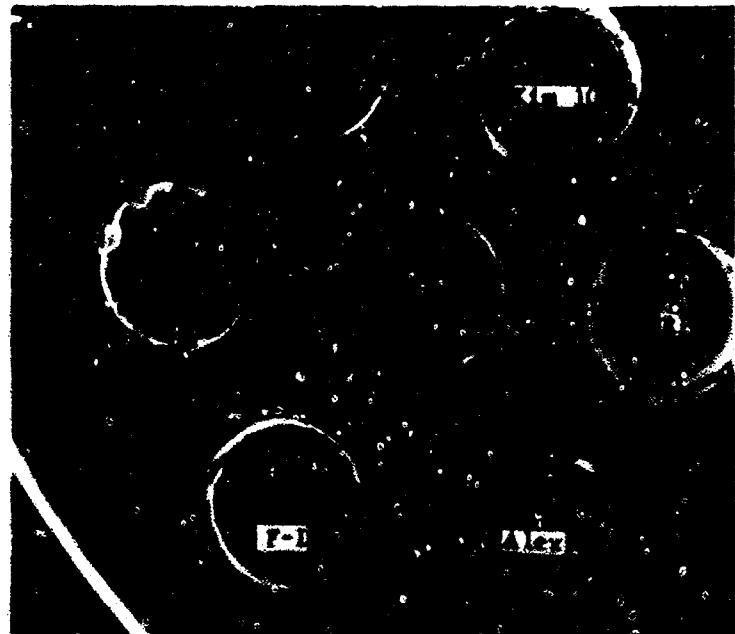


FIGURE 3. Ouchterlony (21) Diffusion Analysis of Serum from One Mouse Immunized with Ribosomes I Protein.



FIGURE 4. Ouchterlony Gel Diffusion Analysis of Six Mice after Immunization with Ethanol-Precipitated Ribosomes.

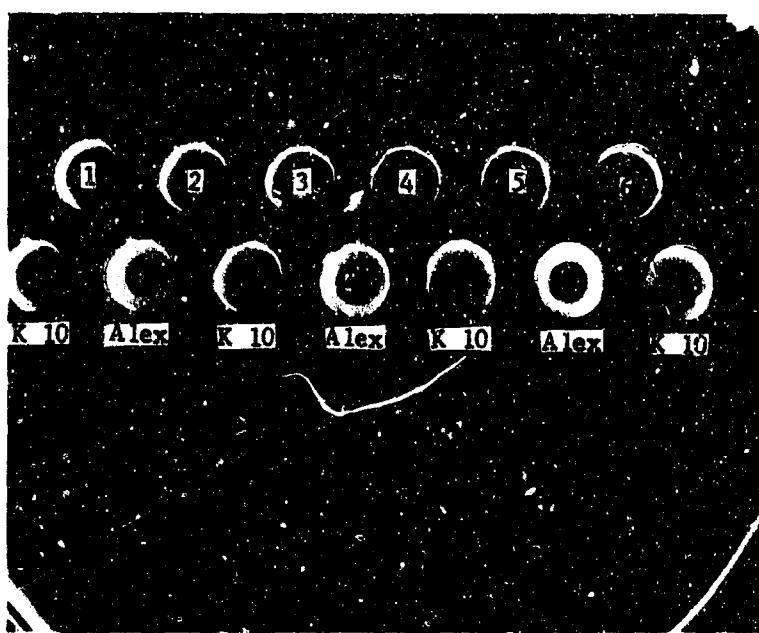


FIGURE 5. Reaction of Sera from Mice Immunized with Ethanol-Precipitated Ribosomes to *P. pestis* Strains Kim 10 and Alexander.

Table 3 shows the results of attempts to immunize animals with phenol-extracted RNA. The surviving animals again had antibodies against Fraction I but failed to produce any antibodies to the RNA preparation.

TABLE 3. RESPONSE OF MICE TO IMMUNIZATION WITH PHENOL-EXTRACTED RIBOSOMAL RNA

Injection Schedule	Dead/ Injected	Antibodies to	
		Phenol-extracted RNA	Fraction I
One Injection (challenged on day 14)	38/40	-	+
One Injection (challenged on day 2)	40/40	0	0
Two Injections 7 days apart (challenged 14 days after second injection)	36/40	-	+
Control	40/40	0	0

In summary, then, the ribosomal protein and RNA isolated from ribosomes of Pasteurella multisp have not proved to be effective in protecting animals against infection with fully virulent strains. Less than 10% of the immunized animals survived the challenge dose.

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